REMARKS

In the Office Action dated 14 August 2003, claims 31-55, all claims pending in the above-captioned U.S. Patent Application were rejected or objected to. Applicants have considered the Action and submit this paper as a full and complete response.

Claims 49 and 55 have been cancelled. Claims 47, 48 and 51-54 have been amended. The now pending claims appear previously.

Applicants' amendments to the claims are believed to obviate the grounds for rejection made on pages 3-5 of the Action.

Claims 31-44 and 55 had been rejected as anticipated by Garini et al., U.S. Patent No. 5,817,462. Claim 55 has been cancelled.

Garini et al. discloses in Example 5 (cf. columns 48 and 49) the fact that a translocation of a small chromosome fragment (a "band") shows up as a band of different color (or pseudo color) in an multicolor FISH/spectral karyotyping hybridization experiment. The well known multicolor FISH/spectral karyotyping technique provides information about inter-chromosomal rearrangements. The chromosome having received a translocated fragment from another chromosome shows an insertion of different color. However, this method does not give any information about the chromosome that has lost the translocated fragment (except for a reduced length of the chromosome that has lost the translocated material, provided that the translocated material is big enough to result in a length reduction that is larger than the random length variation of chromosomes which is due to varying DNA condensation). The loss of material constitutes an intro-chromosomal rearrangement (a deletion) that can not be detected by standard multicolor FISH/spectral karyotyping.

In contrast thereto, the method of the present invention provides detailed information about intra-chromosomal rearrangement like deletions, inversions and duplications.

Further, Garini et al. discloses in Example 6 (cf. columns 49) the method of hybridizing chromosome paints of one species (human) onto chromosomes of another species (gibbon). As the DNA is very similar (but chromosomal localization of genetic material has changed during evolution) this method generates a banding pattern on the target chromosomes. In the limit where paints of all human chromosomes are being labeled with different fluorochrome combinations (e.g. if a human multicolor FISH kit is used) the gibbon chromosomes will exhibit a multi-color banding pattern. This method is limited by the fact that a maximum of 24 different colors (pseudo colors) can be achieved if a multicolor FISH kit is used. In general, the number of distinguishable colors is identical to the number of probes, provided that the probes are distinguishably labeled, e.g. by combinatory labeling (again, the multicolor FISH principle). This limits the applicability of the method for diagnostic applications. It is obvious that e.g. a reciprocal translocation involving two bands of the same color (pseudo color) on the same chromosome or on different chromosomes will not change the color banding pattern and will not be detected. Another limitation is the fact that the color bands generated by cross-species hybridization will not cover the chromosome completely. There will be gaps between at least some of the bands wherein the deletions occurring within these gaps are not detectable.
In contrast thereto, the method of the present invention does not have these limitations, it covers the chromosome without gaps and provides distinguishable color (pseudo color) bands that do not suffer from the above mentioned ambiguity of the cross-hybridization technique.

Moreover, Garini et al. discloses in Example 7 (cf. columns 49 to 51) that dozens of probes may be simultaneously hybridized to chromosomes in metaphase or in interphase, and, if they are labeled distinguishably, the invention of Garini et al. can spectrally detect each of the probes (cf. column49, lines 56 and 57). As in the previous examples the number of detectable fragments/bands/signals is exactly identical to the number of applied (distinguishable labeled) probes.

In contrast thereto, the method of the present invention will generate a number of color bands that is typically 2 to 4 times higher than the number of probes (and fluorochrome combinations) that is hybridized.

Further, Example 8 (cf. column 51 to 52) of Garini et al. discloses the use of probe sets e.g. radiation hybrids of YAC-clones, to generate a color banding pattern or chromosome bar-code. Although, this seems to anticipate the claims of the present invention, the technique presented by Garini et al. is significantly less powerful than the method of the present invention. In Garini et al. a suitable set of probes can be found that hybridize to different loci on a chromosome. If the probes are labeled in distinguishable fluorochrome combinations, the same number of distinguishable color bands can be detected. Example 8 discloses how to generate a bar code consisting of alternating colors. Again the simple combinatorial approach (labeling as well as detection) is quite limited, e.g. translocations involving two color bands of the same color would not be detectable. In order to avoid ambiguities, it is not only necessary to generate a probe set that covers the chromosome without gaps, but each individual probe of the probe set needs to be labeled with a combination of fluorochromes that is different from all the other probes. For practical diagnostic applications very complex probes sets would be required. In contrast thereto, the method of the present invention requires only a few suitable probes (which are partial chromosome paints showing some overlap with the adjacent partial paint) per chromosome. The computer-assisted ratio analysis allows to resolve the color combinations into a number of color bands that is by a factor of 2 to 4 larger than the number of probes employed. Further, the probes can be easily selected to cover the chromosome without gaps. It is, therefore, obvious that the required probe set is relatively simple, and the number of necessary fluorochrome combinations is very limited, which makes the method of the present invention an economic and robust diagnostic method for intra-chromosomal rearrangements. Further, Garini et al. refers to the conventional G- and R-banding (cf. columns 25, lines 53 to 59). However, the present application does not use any conventional banding techniques.

Garini et al. mentions that imaging methods can be helpful in FISH analysis and can provide information beyond pure visual analysis looking down the microscope. Intensity measurements of fluorescence signals may be used in FISH application, e.g. to guess the number of copies of an amplified gene. It is also mentioned that image analysis is used in multicolor FISH (cf. column 26, lines 39 to 44). However, a method to achieve high resolution color banding by combining a relatively small number of partially overlapping probes with ratio analysis is not disclosed and the invention is not anticipated. Rather, the reference supports the need for such a method without suggestions how.

In summary, Garini, et al. does not disclose a method that is based on overlapping combinatorially labeled probes resulting in a multitude of "new" color combinations into pseudo color bands. Garini et al. discloses the standard multicolor FISH method that is able to detect and identify the same number of colors/fragments/probes that was hybridized to the target. Multicolor FISH techniques generally try to avoid an overlap of individual probes resulting in color combinations that do not correspond to any of the individual probes. Overlaps that do occur (e.g. by flaring of adjacent probes, co-localized by a translocation) hamper the analysis rather than providing additional information.

In contrast to multicolor FISH the method of the present invention relies on the overlap of appropriately designed probes. The overlaps resulting in a gradually vary color shades are quantified using color ratio analysis and are displayed as a high resolution banding pattern. The essence of the method of the present invention is the fact that is able to achieve a banding resolution that is up to a factor of 4 higher than the number of applied probes. This has not been anticipated by any of the known multicolor FISH techniques or chromosome bar-coding approaches all of which require basically a number of distinguishable labeled probes ("probe resolution") that is identical to the desired "banding" or bar-code resolution, particularly as long as unambiguity is a requirement.

Claims 31 – 43 and 55 have been rejected as anticipated by Mirzabekov et al., U.S. Patent No. 6,458,584 as well as by Shalon et al., Genome Research: 6, 639 (1996). The references will be grouped since the teachings are related to each other but distinguishable from Applicants' disclosure. Claim 55 has been cancelled.

Mirzabekov et al. and Shalon et al. discloses an oligonucleotide microchip technique and a microarry, respectively. Both techniques differ fundamentally from the method of the present invention. In the array techniques the biomolecule/probe that is to be analyzed is isolated, labeled and co-hybridized with a reference molecule/probe labeled with another fluorochrome onto the template. Intensity differences of the two fluorochromes are an indicator of the relative abundance of test probe versus reference probe. When "translated" to a chromosome situation such a method would correspond to a reverse painting or more precisely to a comparative (genomic) hybridization technique. In Mirzabekov et al. and Shalon et al. the biomolecules to be analyzed are processed and take the role of "probes" that are hybridized to appropriate templates.

In contrast thereto, the method of the present invention does not isolate and label the biomolecules that are to be analyzed. Instead, the biomolecules are analyzed *in situ* by hybridizing appropriate probes onto them. Therefore, the disclosure content of Mirzabekov et al. and Shalon et al. does not anticipate the method of the present invention.

The applicant performed the following experiment to illustrate the differences between the prior art and the present application (cf. Annex 1).

The figure schematically shows a chromosome as target molecule which was labeled by *in situ* hybridization with two different sets of labeled detector molecules, of which one was green and the other one was red. The detector molecules are chosen in a way that the intensity profiles gradually rise or decay along the target molecule (red and green intensity profiles shown in the figure). The resulting color of the target molecule (on the left side next to the intensity profiles

in the figure), obtained by the method disclosed in Garini et al., shows a gradual run from red to green. Without evaluation of the intensity ratios, i.e. without detecting the presence and the intensity of labeled detector molecules at selected regions, a green, a red and an orange-yellow color band can be distinguished wherein the change of the colors is running. A higher resolution is not possible according to Garini et al.

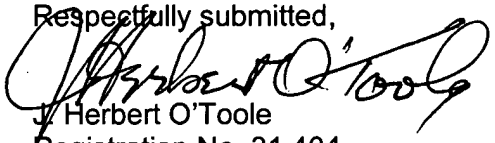
Applicants reserve the right to present the Annex and explanation as a Rule 132 Declaration if future circumstances warrant.

Claims 31-46 had been rejected under 35 USC 103(a) over Garini in view of Cabib, U.S. Patent No. 5,784,162.

The distinctions over Garini have been stated above. Cabib is cited for use of "calibrating dyes." Calibration of dyes long precedes fluorescent microscopy. The fact of calibration does not change the fact that there are more fundamental issues, addressed above, which distinguish the invention from Garini.

In view of the amendment and remarks above, applicant submits that the application is in condition for allowance and requests reconsideration and favorable action.

Respectfully submitted,



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January 14, 2004

Date

Enclosures: Annex
Petition for 2 Month Extension of Time (small)